**PATENT** 

#### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:

SAVERIO CARL FALCO ET AL. CASE NO.: BB1126 USDIV

APPLICATION NO.: UNKNOWN GROUP ART UNIT: UNKNOWN

FILED: HEREWITH EXAMINER: UNKNOWN

FOR: PLANT BRANCHED-CHAIN AMINO ACID

**BIOSYNTHETIC ENZYME** 

# PRELIMINARY AMENDMENT

Commissioner for Patents Washington, DC 20231

Sir:

Prior to examination, please amend the captioned application as follows and consider the following remarks.

#### IN THE SPECIFICATION:

# Please replace the following paragraphs:

#### Paragraph beginning at page 1, line 3:

This application is a divisional application of U.S. Application No. 09/173,300, filed October 15, 1998, which claims the benefit of U.S. Provisional Application No. 60/063,423, filed October 28, 1997.

# Paragraph beginning at page 4, line 7:

Figure 2 (A-C) depicts the amino acid sequence alignments between the dihydroxyacid dehydratase from corn clone cr1.pk0032.c4 (SEQ ID NO:2), soybean contig assembled from clones se3.pk0006.g4, and ses9c.pk001.o8 (SEQ ID NO:4), wheat clone wkm2c.pk005.c12 (SEQ ID NO:6), and *Saccharomyces cerevisiae* (NCBI gi Accession No. 1170543, SEQ ID NO:7). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

#### Paragraph beginning at page 4, line 13:

Figure 3 (A-C) depicts the amino acid sequence alignments between the branched chain amino acid transaminase from corn clone cc71se-b.pk0008.b5 (SEQ ID NO:9), corn clone cen6.pk0003.b5 (SEQ ID NO:11), corn clone cta1n.pk0070.e7 (SEQ ID NO:13), rice clone rls24.pk0025.f6 (SEQ ID NO:15), soybean clone ses8w.pk0032.e9 (SEQ ID NO:17), wheat clone wlm96.pk027.n2 (SEQ ID NO:19), and *Bacillus subtilis* (NCBI gi Accession No. 1706292, SEQ ID NO:20). Amino acids which are conserved among all sequences are

Application No.: Unknown
Docket No.: BB1126 USDIV
Page 2

indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

# Paragraph beginning at page 4, line 21:

Figure 4 (A-B) depicts the amino acid sequence alignments between the branched chain amino acid transaminase from rice clone rls72.pk0014.a3 (SEQ ID NO:22), soybean clone sre.pk0001.d1 (SEQ ID NO:24), soybean clone srr2c.pk003.d20 (SEQ ID NO:26), wheat clone wl1n.pk0123.c11 (SEQ ID NO:28), and *Methanococcus jannaschii* (NCBI gi Accession No. 124380, SEQ ID NO:29). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

## Paragraph beginning at page 4, line 28:

Figure 5 (A-B) depicts the amino acid sequence alignments between the branched chain amino acid transaminase from corn clone cco1.pk0030.d2 (SEQ ID NO:31), wheat clone wkm1c.pk0004.c7 (SEQ ID NO:33), and *Escherichia coli* (NCBI gi Accession No. 1705437, SEQ ID NO:34). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

# Paragraph beginning at page 4, line 34:

Figure 6 (A-C) depicts the amino acid sequence alignments between the *leuC* subunit of 3-isopropylmalate dehydratase from the corn contig assembled from clones cen1.pk0032.b1, cta1n.pk0077.c7, cen3n.pk0015.g3, cen3n.pk0060.h4, and cen3n.pk0121.h11 (SEQ ID NO:36), corn clone cr1n.pk0153.e9 (SEQ ID NO:38), rice clone rl0n.pk087.k16 (SEQ ID NO:40), soybean contig assembled from clones sdp3c.pk008.k13, and srm.pk0006.h5 (SEQ ID NO:42), wheat clone wre1n.pk0045.b10 (SEQ ID NO:44), and *Rhizomucor pusillus* (NCBI gi Accession No. 1708799, SEQ ID NO:45). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

#### Paragraph beginning at page 5, line 5:

Figure 7 (A-B) depicts the amino acid sequence alignments between the *leuD* subunit of 3-isopropylmalate dehydratase from corn clone cr1n.pk0123.b7 (SEQ ID NO:47), rice clone rls12.pk0001.c2 (SEQ ID NO:49), soybean clone srr1c.pk003.c2 (SEQ ID NO:51), wheat clone wl1n.pk0048.a6 (SEQ ID NO:53), and *Lactococcus lactis* (NCBI gi Accession No. 400187, SEQ ID NO:54). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

# IN THE CLAIMS:

Please cancel claims 1-26. Please add the following claims:

27. "added" An isolated polynucleotide comprising:

,

Application No.: Unknown
Docket No.: BB1126 USDIV
Page 3

(a) a nucleotide sequence encoding a polypeptide having leuD activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:47, 49, 51, or 53 have at least 80% sequence identity based on the Clustal alignment method, or

- (b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.
- 28. "added" The polynucleotide of Claim 27 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:47, 49, 51, or 53 have at least 90% sequence identity based on the Clustal alignment method.
- 29. "added" The polynucleotide of Claim 27 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:47, 49, 51, or 53 have at least 95% sequence identity based on the Clustal alignment method.
- 30. "added" The polynucleotide of Claim 27 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:47, 49, 51, or 53.
- 31. "added" The polynucleotide of claim 27 wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:46, 48, 50, or 52.
  - 32. "added" A vector comprising the polynucleotide of Claim 27.
- 33. "added" A recombinant DNA construct comprising the polynucleotide of Claim 27 operably linked to a regulatory sequence.
- 34. "added" A method for transforming a cell comprising transforming a cell with the polynucleotide of Claim 27.
  - 35. "added" A cell comprising the recombinant DNA construct of Claim 33.
- 36. "added" A method for producing a plant comprising transforming a plant cell with the polynucleotide of Claim 27 and regenerating a plant from the transformed plant cell.
  - 37. "added" A plant comprising the recombinant DNA construct of Claim 33.
  - 38. "added" A seed comprising the recombinant DNA construct of Claim 33.
- 39. "added" An isolated polynucleotide comprising a first nucleotide sequence, wherein the first nucleotide sequence contains at least 30 nucleotides, and wherein the first nucleotide sequence is comprised by another polynucleotide, wherein the other polynucleotide includes:
- (a) a second nucleotide sequence, wherein the second nucleotide sequence encodes a polypeptide having leuD activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:47, 49, 51, or 53 have at least 80% sequence identity based on the Clustal alignment method, or
- (b) the complement of the second nucleotide sequence, wherein the complement and the second nucleotide sequence contain the same number of nucleotides and are 100% complementary.

40. "added" A method for isolating a polypeptide encoded by the polynucleotide of Claim 27 comprising isolating the polypeptide from a cell containing a recombinant DNA construct comprising the polynucleotide operably linked to a regulatory sequence.

# ACOMY FUE . A STOC

#### **REMARKS**

Claims 1-26 have been canceled and claims 27-40 added. The newly added claims are drawn to the invention of Group VII (nucleic acid encoding leuD subunit of 3-isopropylmalate dehydratase (hydro-lyase) and host cell) of the restriction requirement mailed March 29, 2000 in the parent application, U.S. Application No. 09/173,300. No new matter is added by the addition of claims 27-40.

Entry of the amendments and favorable consideration of the claims are respectfully requested.

Respectfully submitted,

PAUL D. GOLIAN

ATTORNEY FOR APPLICANTS REGISTRATION NO. 42,591

TELEPHONE: (302) 992-3749 FACSIMILE: (302) 892-1026

Dated: December 20, 2001

Application No.: Unknown
Docket No.: BB1126 USDIV
Page 6

#### VERSION WITH MARKINGS TO SHOW CHANGES MADE

In showing the changes, deleted material is shown as brackets, and inserted material is shown underlined.

#### IN THE SPECIFICATION:

### Paragraph beginning at page 1, line 3:

This application is a divisional application of U.S. Application No. 09/173,300, filed October 15, 1998, which claims the benefit of U.S. Provisional Application No. 60/063,423, filed October 28, 1997.

# Paragraph beginning at page 4, line 7:

Figure 2 (A-C) depicts the amino acid sequence alignments between the dihydroxyacid dehydratase from corn clone cr1.pk0032.c4 (SEQ ID NO:2), soybean contig assembled from clones se3.pk0006.g4, and ses9c.pk001.o8 (SEQ ID NO:4), wheat clone wkm2c.pk005.c12 (SEQ ID NO:6), and *Saccharomyces cerevisiae* (NCBI gi Accession No. 1170543, SEQ ID NO:7). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

# Paragraph beginning at page 4, line 13:

Figure 3 (A-C) depicts the amino acid sequence alignments between the branched chain amino acid transaminase from corn clone cc71se-b.pk0008.b5 (SEQ ID NO:9), corn clone cen6.pk0003.b5 (SEQ ID NO:11), corn clone cta1n.pk0070.e7 (SEQ ID NO:13), rice clone rls24.pk0025.f6 (SEQ ID NO:15), soybean clone ses8w.pk0032.e9 (SEQ ID NO:17), wheat clone wlm96.pk027.n2 (SEQ ID NO:19), and *Bacillus subtilis* (NCBI gi Accession No. 1706292, SEQ ID NO:20). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

# Paragraph beginning at page 4, line 21:

Figure 4 (A-C) depicts the amino acid sequence alignments between the branched chain amino acid transaminase from rice clone rls72.pk0014.a3 (SEQ ID NO:22), soybean clone sre.pk0001.d1 (SEQ ID NO:24), soybean clone srr2c.pk003.d20 (SEQ ID NO:26), wheat clone wl1n.pk0123.c11 (SEQ ID NO:28), and *Methanococcus jannaschii* (NCBI gi Accession No. 124380, SEQ ID NO:29). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

# Paragraph beginning at page 4, line 28:

Figure 5 (A-B) depicts the amino acid sequence alignments between the branched chain amino acid transaminase from corn clone cco1.pk0030.d2 (SEQ ID NO:31), wheat clone wkm1c.pk0004.c7 (SEQ ID NO:33), and *Escherichia coli* (NCBI gi Accession

Application No.: Unknown
Docket No.: BB1126 USDIV
Page 7

No. 1705437, SEQ ID NO:34). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

# Paragraph beginning at page 4, line 34:

Figure 6 (A-C) depicts the amino acid sequence alignments between the *leuC* subunit of 3-isopropylmalate dehydratase from the corn contig assembled from clones cen1.pk0032.b1, cta1n.pk0077.c7, cen3n.pk0015.g3, cen3n.pk0060.h4, and cen3n.pk0121.h11 (SEQ ID NO:36), corn clone cr1n.pk0153.e9 (SEQ ID NO:38), rice clone rl0n.pk087.k16 (SEQ ID NO:40), soybean contig assembled from clones sdp3c.pk008.k13, and srm.pk0006.h5 (SEQ ID NO:42), wheat clone wre1n.pk0045.b10 (SEQ ID NO:44), and *Rhizomucor pusillus* (NCBI gi Accession No. 1708799, SEQ ID NO:45). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

# Paragraph beginning at page 5, line 5:

Figure 7 (A-B) depicts the amino acid sequence alignments between the *leuD* subunit of 3-isopropylmalate dehydratase from corn clone cr1n.pk0123.b7 (SEQ ID NO:47), rice clone rls12.pk0001.c2 (SEQ ID NO:49), soybean clone srr1c.pk003.c2 (SEQ ID NO:51), wheat clone wl1n.pk0048.a6 (SEQ ID NO:53), and *Lactococcus lactis* (NCBI gi Accession No. 400187, SEQ ID NO:54). Amino acids which are conserved among all sequences are indicated with a plus sign (+) while those conserved only within the plant sequences are indicated by an asterisk (\*).

#### IN THE CLAIMS:

Claims 1-26 canceled.

Claims 27-40 added.